

## **A new, sibling species of cave flatworm from Switzerland (Platyhelminthes, Tricladida, Dendrocoelidae)**

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**A new, sibling species of cave flatworm from Switzerland (Platyhelminthes, Tricladida, Dendrocoelidae).** - The paper describes a new species of *Dendrocoelum*, collected from a Swiss cave, representing the second species of this genus to be reported from subterranean localities in Switzerland. The new species closely resembles two other species, *D. clujanum* and *D. stenophallus*, both from Roumania.

**Keywords:** Platyhelminthes - Tricladida - Dendrocoelidae - *Dendrocoelum nekoum* sp. nov. - new species - sibling species - Switzerland.

### INTRODUCTION

The most recent and comprehensive review of European subterranean planarians (Platyhelminthes, Tricladida) is that of Goubault (1972). A more recent summary of the geographic distribution of the various species was published by the same worker (Goubault, 1994). The only subterranean planarian mentioned for Switzerland is *Dendrocoelum infernale* (Steinmann, 1907), which was reported from caves, springs, and the profundal habitat of lakes in three cantons, viz. Berne, Saint-Gall, and Schwyz (Goubault, 1972). The present paper describes the finding of another, new member of the genus *Dendrocoelum* from a fourth canton, Obwalden.

The genus *Dendrocoelum* Örsted, 1844 s.l. comprises about 80 nominal species and has a principally European distribution, which extends to the region of the Caspian Sea and also includes northern Africa (cf. Harrath *et al.*, 2012).

### MATERIAL AND METHODS

The animals were fixed in Bouin's fluid and, subsequently, transferred to 70% ethanol. Specimens were embedded in paraffin (histowax); histological sections were made at intervals of 8 µm and were stained in Mallory-Cason. Reconstructions of the copulatory apparatus were obtained using a camera lucida attached to a compound microscope. The material is deposited in the collections of the Netherlands Centre for Biodiversity Naturalis (section ZMA).

*Abbreviations used in the figures:* ad, adenodactyl; ab, adenodactyl bulb; ap, adenodactyl papilla; bc, bursal canal; ca, common atrium; cb, copulatory bursa; cod,

common oviduct; fl, flagellum; go, gonopore; ma, male atrium; od, oviduct; ph, pharynx; pb, penis bulb; pp, penis papilla; sg, shell glands; vd, vas deferens

## SYSTEMATIC ACCOUNT

**Order Tricladida Lang, 1884**

**Family Dendrocoelidae Hallez, 1892**

**Genus *Dendrocoelum* Örsted, 1844**

*Dendrocoelum nekoum* sp. nov.

**MATERIAL EXAMINED:** Holotype: ZMA V.Pl. 6903.1, Riedschwandhöhle, 6078 Lungern, Klein Melchtal, 46°47' N 8°13'E, Switzerland, alt. 1550 m, a small lake about 100 m into the cave, 22 March 2009, sagittal sections on 17 slides. Paratype: V.Pl. 6903.2, *ibid.*, sagittal sections on 12 slides.

**ETYMOLOGY:** The specific epithet is derived from the acronym NeKO for the foundation "Naturerbe Karst und Höhlen Obwalden", the organization that has as one of its members the collector of the material, viz. Martin Trüssel.

**DIAGNOSIS:** *Dendrocoelum nekoum* can be distinguished from its congeners by the following combination of features: absence of eyes; long penial papilla, provided with a spacious lumen, with at its tip an inflexible flagellum; ovaries positioned at about 1/4<sup>th</sup> of the distance between the brain and the root of the pharynx; testes extending from about 1/2 of the distance between the brain and the root of the pharynx to the level of the gonopore; adenodactyl with the so-called Balkan type of musculature.

**DESCRIPTION:** Preserved specimens 12x4 mm; live animals were estimated to measure about 15 mm in length. Anterior end truncated and provided with a small, cup-shaped sucker. Animals devoid of any pigmentation and eyes (Fig. 1).

Pharynx small, located in the posterior half of the animal, measuring about 1/9<sup>th</sup> of the body length; musculature of the dendrocoelid type, i.e. with the inner zone consisting of a layer of intermingled circular and longitudinal muscles. Mouth opening situated at the posterior end of the pharyngeal cavity.

The testes are situated at the dorsal side of the body, as well as in the middle part and at the ventral side; the follicles extend from about one-half of the distance between the brain and the root of the pharynx (i.e. from a considerable distance posterior to the ovaries) to the level of the gonopore. The ovaries are located at about 1/4<sup>th</sup> of the distance between the brain and the root of the pharynx.

The sperm ducts are swollen to spermiducal vesicles but upon penetrating the anterior wall of the penis bulb they decrease considerably in diameter. Whilst traversing the bulb, the vasa deferentia expand again somewhat in diameter and open into the proximal section of the penis lumen. The latter is wide, occupying most of the space of the elongated penis papilla, and at the distal end of the papilla even expands to such an extent that hardly any penial mesenchyme is present (Fig. 2).

The penis papilla occupies most of the male atrium. The tip of the penis papilla is provided with a highly characteristic flagellum. The shape of this flagellum seems to be independent of the degree of extension or contraction of the penis papilla. In contrast to the holotype, the penis papilla of the paratype projects for a considerable

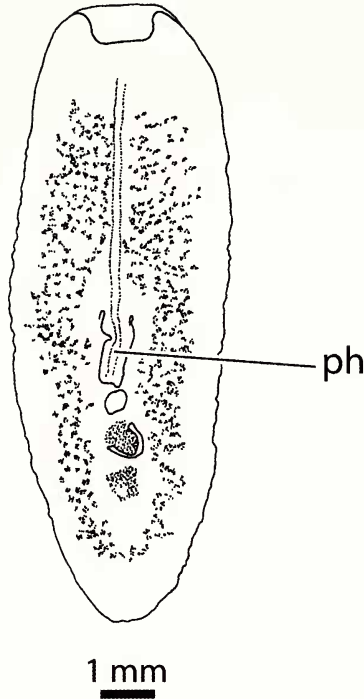


FIG. 1

Cleared specimen of *Dendrocoelum nekoum*, viewed from the ventral side.

distance through the gonopore. However, the shape of the flagellum of the paratype is precisely the same as in the holotype. The lining epithelium of the very tip of the penis, and thus of the flagellum, is pierced by openings of erythrophil penis glands. The penis bulb consists of criss-cross arranged, strong muscle fibres. This mess of circular and longitudinal fibres extends throughout the mesenchyme of the penis papilla, albeit much more faintly developed. The epithelium of the penis papilla is underlain by a well-developed layer of circular muscle.

The two oviducts join to form a common oviduct immediately dorsally to the most posterior section of the male atrium, which receives the opening of the common oviduct at its most postero-dorsal section. The common oviduct and the posteriormost sections of the oviducts receive the secretion of erythrophil shell glands. Oviducts and common oviduct are lined with cuboidal cells; those of the common oviduct are nucleated, while the cells of the oviducts are infranucleated.

A large copulatory bursa is situated directly posterior to the pharyngeal pocket. A long and somewhat undulating bursal canal connects it with the common atrium. The bursal canal is lined with a nucleated epithelium and is surrounded by a well-developed subepithelial layer of circular muscles, followed by a thin layer of longitudinal muscles (Fig. 2).

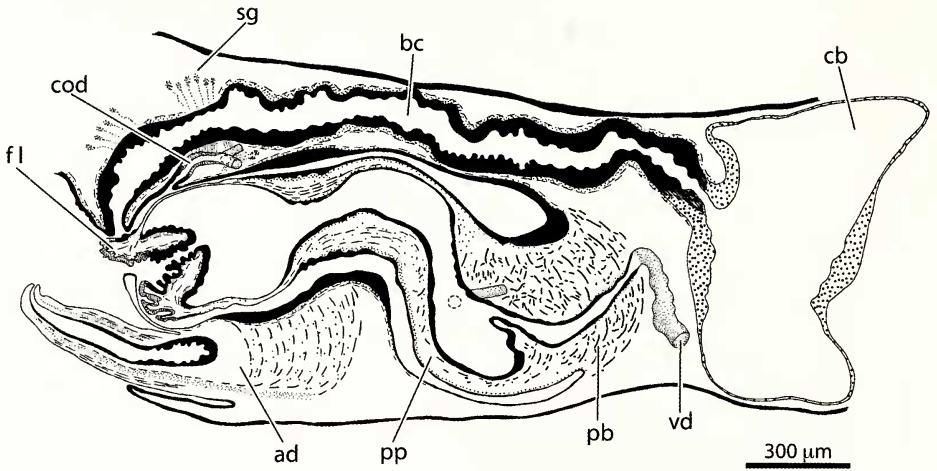


FIG. 2

*Dendrocoelum nekoum*. Holotype. Sagittal reconstruction of the copulatory apparatus.

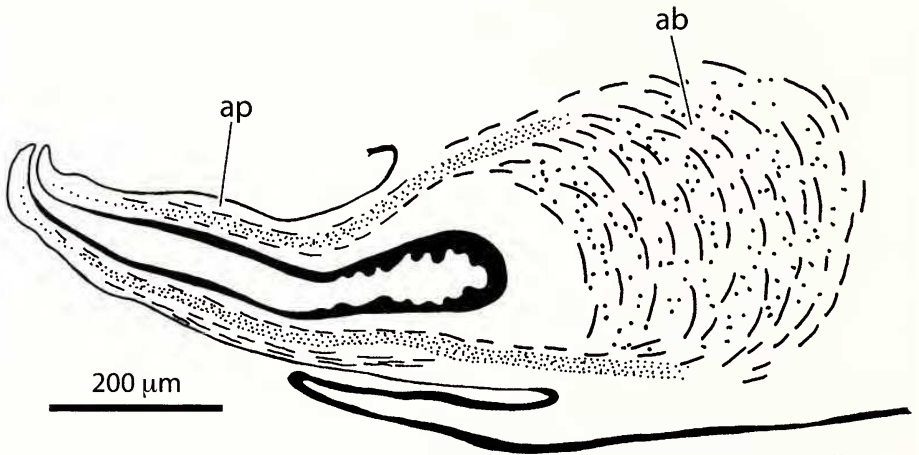


FIG. 3

*Dendrocoelum nekoum*. Holotype. Sagittal reconstruction of the adenodactyl.

A well-developed musculo-glandular organ or adenodactyl is located underneath the posterior half of the male atrium. This adenodactyl consists of a highly muscular bulb, consisting of strong, intermingled layers of muscle fibres, and a conical papilla (Figs 2, 3). The papilla is provided with a distinct lumen and its mesenchyme is traversed by a relatively thick, pale blue staining layer of circular muscle fibres that is both dorsally and ventrally bounded by a single layer of longitudinal muscle fibres. This condition is similar to the Balkan type of adenodactyl recently described by Harrath *et al.* (2012).

DISCUSSION: The gross morphology of *D. nekoum*, and in particular its characteristic penial flagellum, resembles only three other species of *Dendrocoelum*: *D. her-*

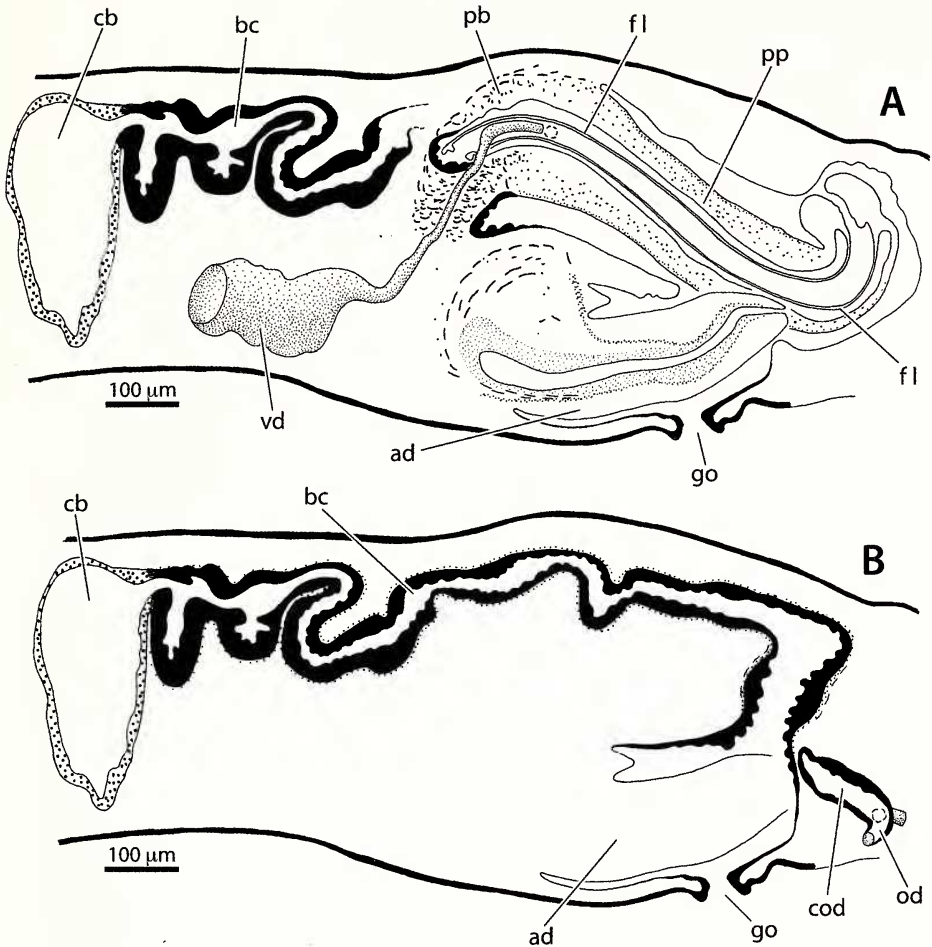


FIG. 4

*Dendrocoelum hercynicum*. Syntype (Museum für Naturkunde 10436b). (A) Sagittal reconstruction of the copulatory apparatus. (B) Sagittal reconstruction of the copulatory bursa and bursal canal; exact opening of common oviduct into the atrium could not be observed.

*cynicum* Flössner, 1959; *D. clujanum* Codreanu, 1943; *D. stenophallus* Codreanu & Balcesco, 1967.

*Dendrocoelum hercynicum* from Sachsen (Germany) has been described with a very long, inverted penial flagellum, the tip of the latter being swollen and thus suggesting a structure similar to the flagellum of *D. nekoum* (cf. Flössner, 1959, fig. 4). For comparative studies I have sectioned a syntype specimen of *D. hercynicum*, since no histological material of this species was available. Examination of this specimen confirmed the presence of an extremely long and inverted flagellum, albeit that the tip did not show the swelling as depicted by Flössner (Fig. 4A). Evidently, this flagellum

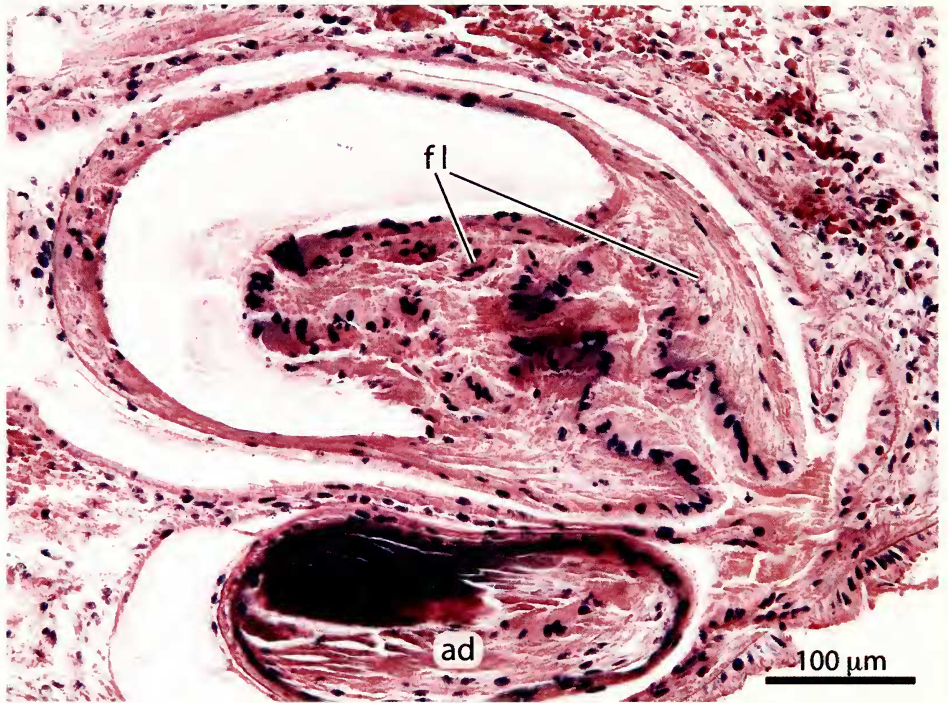


FIG. 5

*Dendrocoelum stenophallus* (MNHN AJ 275-276). Microphotograph of flagellum on the penis papilla.

is highly different from the short one in *D. nekoum*. Furthermore, the bursal canal of *D. hercynicum* shows many lateral undulations, as noticed also by Flössner (1959, fig. 4), which are absent in *D. nekoum* (Fig. 4B).

After examination of the descriptions of *D. clujanum* and *D. stenophallus*, both from Roumania, one can only conclude that these nominal species are very similar, if not the same. The only structural difference concerns the solely dorsal testes in *D. clujanum* (Codreanu, 1943) and the situation in *D. stenophallus* in which the testes occur throughout the dorso-ventral space. Both species have an elongated penis papilla provided with a distally distinctly widened penial lumen. In addition, the tip of the penis is provided with a swelling resembling the flagellum of *D. nekoum*. Unfortunately, the type material of both *D. clujanum* and *D. stenophallus* was not available for study. However, I have been able to re-examine paratypes of *Dendrocoelum dimitrescuae* Gourbault, 1968, a junior synonym of *D. stenophallus*, the material of which was collected from the same cave as *D. stenophallus*.

The microphotographs in Gourbault (1967, plate II, E) already suggested a penis structure, notably a flagellum, very similar to that of *D. nekoum* (Fig. 5). Gourbault (1967, p. 812) doubted whether this flagellum could be inverted, due to a lack of sufficient musculature. My observation on *D. nekoum* (see above) suggests indeed a non-inversible or non-eversible flagellum.

Codreanu & Balcesco (1967) described for *D. stenophallus* the presence of a muscular sphincter between male and common atrium. However, specimen AJ 275-276 shows no sphincter in this region. It is only the case that the circular musculature around the vaginal region of the bursal canal is somewhat more developed than on other parts of the canal, a condition that was also described by Gourbault (1967, p. 810).

A difference between *D. nekoum* and *D. stenophallus* resides in the distribution of the testes along the axis of the body. In *D. stenophallus* the anteriormost testes are also far removed from the brain and the ovaries, i.e. located at one-half of the distance between the brain and the root of the pharynx, similar to the condition in *D. nekoum*. However, in *D. stenophallus* the follicles extend to the posterior end of the body, whereas in *D. nekoum* they reach to the level of the gonopore.

Another difference concerns the position of the ovaries. In *D. stenophallus* the ovaries are located at about 1/8<sup>th</sup> of the distance between the brain and the root of the pharynx, whereas in *D. nekoum* the gonads are situated at 1/4<sup>th</sup> of this distance. For *D. clujanum* it is also described that the testes extend into the posterior end of the body (Codreanu, 1943).

In view of these small differences and the fact that the subterranean Roumanian localities of both *D. clujanum* and *D. stenophallus* are far removed and, presumably, completely isolated from Swiss caves, it is here proposed that *D. nekoum* represents a new species. The geographic argument lends some strength from the observation that subterranean forms, in general, have a restricted distribution (Gourbault, 1994). Thus, the new species forms the third member of a group of sibling species of which the two other members are *D. stenophallus* and *D. clujanum*.

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